Selectivity of TRAIL-mediated apoptosis of cancer cells and synergy with drugs: The trail to non-toxic cancer therapeutics (Review)

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Abstract. There have been many advances in the therapy of cancer following the introduction of cytotoxic chemotherapeutic drugs. Notable responses were observed in primary tumors and often in malignant metastatic tumors. However, one of the consequences of chemotherapy is the development/acquisition of drug-resistant phenotypes and the development of multiple drug resistance. The development of drug resistance remains a major obstacle in the treatment of such tumors and therefore, there is an obvious need for alternative approaches such as immune/gene therapy. The cloning of biologically active cytotoxic molecules has been considered as potential new therapeutics in the destruction of drug-resistant tumor cells. For instance, some members of the TNF-superfamily are characterized by their ability to inflict cell death upon binding to their cognate receptors. TNF-α was the first molecule to be tested for its anti-tumor activity, followed by Fas-ligand. These two molecules are efficient in killing a variety of tumor cells, however, they cause significant damage to normal tissues that result in life-threatening toxicities. Therefore, the search for a cytotoxic molecule that is selective for tumor cells has continued until the recently discovered new member of the TNF superfamily, namely TRAIL/APO-2L. TRAIL has been shown to be selectively cytotoxic in inducing apoptosis against tumor cells and has minimal or no toxicity against normal tissues, as examined both *in vitro* and *in vivo* in mice. Therefore, TRAIL is a new agent that has great potential for its *in vivo* anti-cancer effect, whether used alone or in combination with drugs. Studies from our laboratory have recently demonstrated that tumor cells that are resistant to TRAIL can be sensitized by subtoxic concentrations of drugs/cytokines and the sensitized tumor cells are significantly killed by TRAIL. This review describes the current status of research studies performed with TRAIL by other investigators as well as by our laboratory.

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Key words: TRAIL/APO-2L, apoptosis, sensitization, synergy, therapy, prostate carcinoma, myeloma, bladder cancer, Kaposi's sarcoma

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1. Introduction

Apoptosis, or programmed cell death (PCD), is a genetically controlled response for cells to commit suicide. The symptoms of apoptosis are viability loss accompanied by cytotoxic boiling, chromatin condensation, and DNA fragmentation (1). What has pushed apoptosis into the forefront of cancer research has been the identification of genes that control cell death and the appreciation of the role of apoptosis in development and disease. Some gene products are activators of apoptosis, whereas others are inhibitors. The characterization of these gene products will help define the process of cell death at the biochemical level and the development of new drugs that can selectively modulate genes regulating apoptosis.

Apoptosis is a mode of cell death in which single cells are killed in the midst of living tissue. Apoptosis accounts for most or all of the PCD responsible for tissue modeling in vertebrate development for the physiological cell death in the course of normal tissue turnover. Apoptosis is also responsible for the extensive elimination of cells of the B and T cell lineages during negative selection in the immune response. Irradiation, chemotherapy and the appropriate hormone therapy all induce apoptosis in tumor cells, although high doses may also cause cell destruction by other means (2-9).

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1. Introduction

Apoptosis, or programmed cell death (PCD), is a genetically controlled response for cells to commit suicide. The symptoms of apoptosis are viability loss accompanied by cytotoxic boiling, chromatin condensation, and DNA fragmentation (1). What has pushed apoptosis into the forefront of cancer research has been the identification of genes that control cell death and the appreciation of the role of apoptosis in development and disease. Some gene products are activators of apoptosis, whereas others are inhibitors. The characterization of these gene products will help define the process of cell death at the biochemical level and the development of new drugs that can selectively modulate genes regulating apoptosis.

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The development of defects in PCD mechanisms can extend the life span of a cell and can contribute to neoplastic cell expansion. Also, defects in PCD can contribute to carcinogenesis by permitting genetic instability and accumulation of gene mutations promoting resistance to immune-based destruction and conferring resistance to cytotoxic drugs and radiation. These manifestations indeed are seen in malignant cells not responding to these therapies. There have been several reviews that describe the progress in the molecular and biochemical control of apoptosis and tumor cell developments to block apoptosis. These advances should reveal new therapeutic strategies to fight cancer by restoring their sensitivity to apoptosis (10,11).

There have been many advances in the therapy of cancer following the introduction of cytotoxic chemotherapeutic drugs. Notable responses were observed in primary tumors and often in malignant metastatic tumors. However, one of the consequences of chemotherapy is the development/acquisition of drug-resistant phenotypes and the development of multiple drug resistance. The development of drug resistance remains a major obstacle in the treatment of such tumors and the obvious need for alternative approaches such as immune/gene therapy.

One of the hallmarks of some members of the TNF-superfamily is the induction of cell death by apoptosis upon binding to their cognate receptors. TNF-α was the first molecule to be tested for its anti-tumor activity, followed by Fas-ligand. These two molecules are efficient in killing a variety of tumor cells, however, they cause significant damage to normal tissues that result in life-threatening toxicities (12-14). TRAIL/APO-2L, on the other hand, is selectively cytotoxic to tumor cells and transformed cells and is not cytotoxic to normal tissues (15,16). *In vivo* experiments in mice also show lack of toxicity by TRAIL in normal tissues (17).

This review describes studies reported on the sensitivity of tumor cells to TRAIL. In addition, we report our recent studies on the sensitivity of various tumor cells to TRAIL and their sensitization by cytotoxic drugs. These studies are the beginning for exploring the potential therapeutic effects of TRAIL *in vivo* in the treatment of drug-resistant tumor cells.

2. The TNF superfamily

Table I summarizes the TNF ligand and receptor superfamilies. The TNF family includes neurotrophins, TNF-α, Fas ligand (Fas-L/CD95/Apo-1L), TRAIL/Apo-2L, CD30L, CD40L, CD27, 4-1BBL, OX-40L, and lymphotoxin (LT) α, β. Except for LT α, all ligands are synthesized as type II transmembrane proteins; their N-terminus is in the cytoplasm and their C-terminus extends into the extracellular region (35). A region of about 150 amino acid residues in the extracellular domain is 20-25% homologous among the TNF family members (35). LT α does not possess a membrane-anchoring sequence and is found as a soluble form that can heterotrimerize with a 33 kDa glycoprotein named LT β with α1β2 or α2β1 stoichiometry (36).

The common feature of the ligands is that all active ligands are composed of 3 identical subunits (trimers) and activate their respective receptors by oligomerization (35-37). Although most members are found as membrane-bound molecules, specific metalloproteases are capable of generating soluble forms (35-37). Recently, the cDNA encoding for a zinc-dependent metalloprotease for TNF-α called TACE (TNF-α converting enzyme) has been cloned (36,37).

3. The TNF receptor superfamily

The TNF receptor superfamily includes NTR/GFR (p75), TNF-R2 (CD120b), TNF-R1 (CD120a), Fas (CD95/Apo-1), DR3 (TRAMP/WSL-1), DR4 (TRAIL-R1), DR5 (TRAIL-R2), DcR1 (TRAIL-R3), DcR2 (TRAIL-R4), CD30, CD40, CD27, 4-1BB (CD137), OX-40, LT-βR, human HVEM (herpes virus early mediator), OPG (osteoprotegerin)/OCIF, and RANK (16,35-39). All of the receptors are type I transmembrane proteins with an extracellular region composed of two-six cysteine rich domains (CRDs) that have about 25% identity among members and contribute to ligand binding. Fas, TNF-R1, TRAIL-DR4, DR5, TRAMP (DR3), and CAR1 have similar cytoplasmic domains. Sequence comparison of the intracellular region of these receptors revealed a homologous, well-conserved region of about 80 amino acids called the death domain (DD) (16,35,36). The death domain is absolutely required for the specific recruitment of cellular singling molecules (adaptor proteins) that are implicated in apoptosis (35) (Table I).

4. TRAIL/APO-2L

TRAIL is very similar to Fas ligand in its ability to induce apoptosis. Like FasL, TRAIL can kill many sensitive tumor cell lines in 4-8 h. In contrast, TNF kills tumor cell lines in more than 24 h (40-43). The TRAIL receptors DR4 and DR5, like the full-length Fas receptors, contain a death domain that possibly interacts with an adaptor molecule in order to mediate the apoptotic signal (24,44-47). The identification of the adaptor molecules has been controversial. Some investigators have observed that overexpression of dominant negative FADD (FADD-DN) can inhibit TRAIL-mediated apoptosis suggesting FADD (Fas-associated death domain) is the adaptor molecule responsible for mediating the death signal of TRAIL (24,44,45,48). However, another study using a similar strategy was not able to demonstrate similar results (25,46,47). Nowadays, overexpression studies are accepted as possibly flawed because extremely high amounts of FADD-DN molecules can lead to non-physiological promiscuous association of death domain to the TRAIL receptor. The most convincing evidence is the FADD-deficient mice studies that show that fibroblast cells from these mice remain sensitive to TRAIL-mediated apoptosis (49). Although the involvement of FADD in the TRAIL-mediated apoptosis is not certain (16), the presence of death domain on TRAIL receptor suggests a similar death domain molecule that mediates the apoptotic signal like FADD.

The initiation of TRAIL apoptosis involves the clustering of three DR4 or DR5 on the target cell surface by cross-linking the receptors with the ligand (TRAIL). Upon oligomerization of the receptors, an adaptor molecule similar to FADD is recruited to the DR4 or DR5 receptor cluster via death domain interactions (22). The cross-linking of agonistic receptors DR4 and DR5 to TRAIL can be inhibited by

Table I. TNF ligand and receptor superfamilies.

Ligands	Receptors	Proposed Functions	Selected References
Neurotrophins (NGF, BDNF, NT-3, NT-4)	P75 NTR/NGFR	Neuronal development	(18)
TNF	TNFR2/CD120b	Inflammatory response, Cell death	(19)
	TNFR1/CD120a	Cell death	(19)
FasL	Fas/CD95	Cell death	(20)
	Soluble Fas	Inhibition of cell death	(21)
DR3L/Apo-3L/Tweak	DR3/TRAMP /WSL-1	Cell death	(22)
TRAIL/Apo-2L	DR4/TRAIL-R1	Cell death	(23)
	DR5/TRAIL-R2	Cell death	(24)
	DcR1/TRAIL-R3	Inhibition of cell death	(25)
	DcR2/TRAIL-R4	Inhibition of cell death	(25)
CD30L/CD153	CD30	B/T cell development, Cell death, Differentiation	(26)
CD40L	CD40	B/T cell development, Survival, Differentiation	(27)
CD27	CD27	B/T cell costimulation, Activation, Development	(28)
4-1BBL	4-1BB/CD137	APC/T cell costimulation, T cell survival	(29)
OX40L	OX40	T cell costimulation, Activation, Development	(30)
LTα1β2	LT-βR	Inflammatory response, Antibody production	(31)
LIGHT, LTα	HVEM/ATAR	T cell growth	(32)
TRANCE/RANKL/OPGL	OPG/OCIF	Dendritic cell differentiation, Bone development	(33)
	RANK	Dendritic cell differentiation	(34)

Cysteine rich motif | Transmembrane region | Death domain (DD) | Truncated death domain

the decoy receptors (DcR1 and DcR2) (16,25). The decoy receptors are able to inhibit TRAIL-mediated apoptosis because they lack functional death domain to mediate the death signal and they can compete with the binding to TRAIL by DR4 and DR5 (50). The TRAIL adaptor molecule similar to FADD possibly contains a death effector domain that binds FLICE (caspase-8), the aspartate-specific cysteine protease that initiates a caspase amplification cascade leading to the ultimate apoptotic phenotypes (51). When the adaptor is recruited to the death domain of the TRAIL receptors DR4 or DR5, FLICE zymogen is brought together in close proximity by the FADD-like adaptor and is activated by FLICE auto-cleavage (16,51). The FLICE activating complex that consists of TRAIL receptor-adaptor-FLICE is named as DISC (death inducing signaling complex) (52). The active FLICE enzyme subsequently activates caspase-3 and other caspases by cleaving their zymogen forms (43,53). Active caspase-3 can then cleave ICAD (inhibitor of caspase-activated deoxy-ribonuclease), resulting in the release of active nuclease that cleaves DNA into 180-220 bp fragments, a typical hallmark of apoptosis (54) (Fig. 1).

5. Biological activities of TRAIL

Sensitivity of tumor cell lines to TRAIL. Since its discovery in 1995 (40), the direct cytotoxic effect of soluble recombinant TRAIL on a variety of tumor cells has been the main focus of researchers in the field. A large panel of various tumor cell lines of hematopoietic origin (myeloid, lymphoid, erythroid), cervical carcinoma, lung adenocarcinoma, colon carcinoma, melanoma, renal, breast carcinoma and glioma have been tested for their sensitivity to TRAIL. In numerous *in vitro* studies, most tumor cell lines have been shown to be sensitive to TRAIL-mediated apoptosis (25,39-41,55-60). Several studies have also demonstrated that although non-transformed cells such as keratinocytes (61) and PBL (25,59) express TRAIL

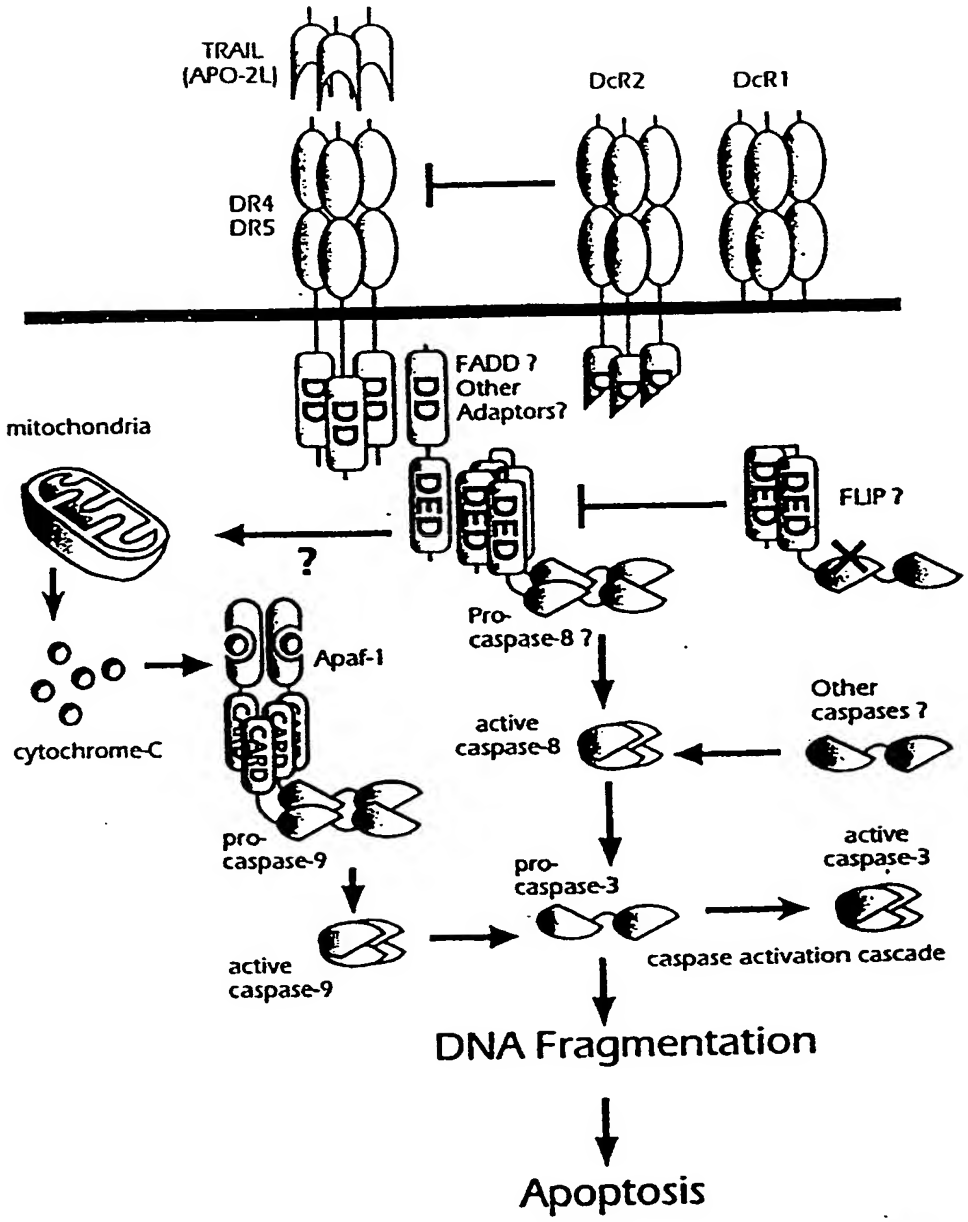

Figure 1. TRAIL-mediated apoptosis signaling pathway. Upon activation of DR4 and DR5, pro-caspase-8 is recruited via interactions with FADD-like adaptor molecules. Decoy receptors DcR1 and DcR2 can inhibit the signal by competitive binding with TRAIL. Once pro-caspase-8 is recruited, it is autocleaved and activated. Active caspase-8 further activates down-stream effector pro-caspases (pro-caspase-3 or other pro-caspases). FLIP, which is structurally similar to pro-caspase-8 but missing a functional caspase catalytic site, can inhibit the activation of pro-caspase-8. Active caspase-3 then causes PARP cleavage and DNA fragmentation. An alternative mitochondrial pathway can possibly be activated. When the mitochondrial pathway is activated, cytochrome C is released and binds to Apaf-1 to activate pro-caspase-9. Active caspase-9 can activate pro-caspase-3 and leads to DNA fragmentation and apoptosis.

and TRAIL receptors, they are not sensitive to the cytotoxic effects of TRAIL.

The *in vivo* tumoricidal activities of TRAIL have been recently documented. TRAIL-sensitive human mammary adenocarcinoma cells (MDA-231) were intraperitoneally (IP) and subcutaneously (SC) implanted into CB.17 SCID mice. Subsequent IP injections of TRAIL prolonged the survival of the mice which were challenged IP and 25% of them were remained alive at the end of the experiment (3 months). TRAIL also profoundly suppressed the tumor growth in SC challenged mice, with no detectable tumor mass in the majority of the cases. The re-occurring tumors also showed sensitivity to TRAIL (17). The potential therapeutic effects of TRAIL in treating tumor-bearing mice was also examined. Intraperitoneal or intravenous TRAIL injections were able to cure SC administered tumors at day 40. High doses of TRAIL

(14 days, 500 μg/day, IP) decreased the size of pre-existing tumors (17), and no detectable toxic side effects were observed.

Involvement of TRAIL in cell-mediated cytotoxic functions. Constitutive or inducible TRAIL expression following T cell activation by PMA/Ionomycin, IL-2, IFN α, ß, or anti-CD3 antibody has been observed in $CD4^+$ and $CD8^+$ T cells (53,60,62). Functional constitutive TRAIL expression was also seen on primary NK cells (63) as well as on murine and human activated B and T cells (64). Constitutive TRAIL expression was detected on some melanoma specific, HLA-A1 restricted $CD4^+$ clones (55). These data suggest an involvement of TRAIL in T cell-mediated cytotoxicity, which might complement the apoptotic activities of Fas and perforin/granzyme pathways (53,55,59,60,62-64).

Table II. TRAIL receptor expression in multiple myeloma cell lines.

Cell line	Expression of TRAIL receptor mRNA[a]				Sensitivity to TRAIL killing[b]
	DR4	DR5	DcR1	DcR2	
8226	Strong	Weak	Weak	Strong	Sensitive
8226/Dox 40	Strong	Weak	Weak	Strong	Sensitive

[a]Receptor expression was determined by RT-PCR. The level of expression of receptor mRNA was compared to the mRNA level of GAPDH. (Weak indicates 5-50% of GAPDH; and Strong indicates 50-100% of GAPDH). [b]Sensitive at concentrations of 0.01-10 ng/ml.

6. TRAIL-resistant tumor cells and their sensitivity to TRAIL-mediated apoptosis

The selective cytotoxicity of TRAIL-mediated apoptosis on tumor cells, but not normal cells, its involvement in T cell-mediated cytotoxicity, plus the absence of toxic side effects upon *in vivo* administration, have made TRAIL an attractive drug for resistant tumor cells and a selectively cytotoxic agent for tumor therapy, particularly in tumors that are sensitive to TRAIL.

However, the major hurdle in treating cancer is the development of resistant tumor cells to drugs and the development of anti-apoptotic machinery which can spell over TRAIL sensitivity to apoptosis. This led to a number of studies which demonstrated the synergistic effects of a combination of subtoxic concentrations of chemotherapeutic drugs and TRAIL on TRAIL-resistant tumor cells (65).

Effect of cycloheximide. For example, the addition of cycloheximide (CHX) or Actinomycin D (Act D) on TRAIL-resistant melanoma cell lines has been shown to reverse their resistance to TRAIL-mediated apoptosis (15,55). In a series of studies, the majority of a large panel of glioma cell lines showed sensitivity to TRAIL. However, the addition of cycloheximide (CHX) enhanced TRAIL-induced cytotoxicity particularly at low concentrations (57). Doxorubicin is also capable of sensitizing TRAIL-resistant human breast carcinoma cells (66). We have also shown the reversal of TRAIL resistance in several tumor systems by other drugs as will be discussed briefly below.

Effect of other drugs (e.g. Act D, ADR, CDDP)

i) Multiple myeloma. MM cells are resistant to currently available therapeutic modalities such as chemotherapy (vincristine-dexamethasone-doxorubicine and melphalan-prednisone), radiotherapy, autologous bone marrow transplantation, and stem cell transplantation (67,68). While MM cells may initially respond to conventional anti-tumor therapeutic approaches (combined chemotherapy and radiation therapy), almost all patients suffer from relapse (67). This relapse is due to a selective outgrowth of a subpopulation of tumor cells that develop resistance to drugs. It has been proposed that tumor cells not only develop resistance to the drugs that has been initially used in the treatment, but also develop a cross-resistance to other structurally unrelated therapeutic modalities. The development of tumor cell drug resistance in patients with malignancy has led to the exploration of alternative therapeutic approaches such as immunotherapy (65). The ultimate goal is to achieve complete regression of the tumor by overcoming their resistance to apoptosis-mediated stimuli in the absence of tissue toxicity.

We have carried out studies on the effects of TRAIL on multiple myeloma. The 8226/Dox 40 multiple myeloma cell line is derived from the drug-sensitive RPMI 8226/S parental cell line by continuous exposure to stepwise increasing concentrations of doxorubicin (10-400 nM). This line is a useful model system for studying multidrug resistance (69). RPMI 8226/S and 8226/Dox 40 cell lines express Fas (CD95/Apo-1) as detected by RT-PCR and FACS analysis (data not shown). However, despite the expression of Fas on these cells, they are resistant to the cytotoxic effects of PMMI (murine CTL hybridoma) cells which solely kill through Fas/Fas ligand pathway in short-term cytotoxicity assays, as well as anti-Fas monoclonal antibody (CH-11). This resistance might be due to mutations in the Fas cytoplasmic death domain (70), presence of soluble Fas which may interfere with Fas/Fas ligand interactions (71), a protective signal from CD38 antigen (71), or the expression of anti-apoptotic molecules such as Bcl-2 and Bcl-x_L (72). This led us to the investigation of the apoptotic capability of soluble TRAIL on drug-resistant/Fas-resistant multiple myeloma cell lines. Both the RPMI 8226, and 8226/Dox 40 cells express TRAIL receptors DR4 (R1), DR5 (R2), DcR1 (R3), and DcR2 (R4) as detected by RT-PCR (Table II).

We initially observed that multiple myeloma cell lines are sensitive to very low concentrations (>1 ng/ml) of TRAIL. The cytotoxic effects of TRAIL on murine multiple myeloma cell lines was also shown by other groups (64). The 8226/S cell line is sensitive to adriamycin (ADR) at concentrations of 0.1, 0.5 and 1 μg/ml, whereas the 8226/Dox 40 is resistant. As shown in Fig. 2, both of these cell lines show synergistic responsiveness to low concentrations of combined ADR and TRAIL. We have also demonstrated the synergistic cytotoxicity of etoposide (VP-16) and TRAIL on these cells (data not shown). To our knowledge, this is the first study that shows

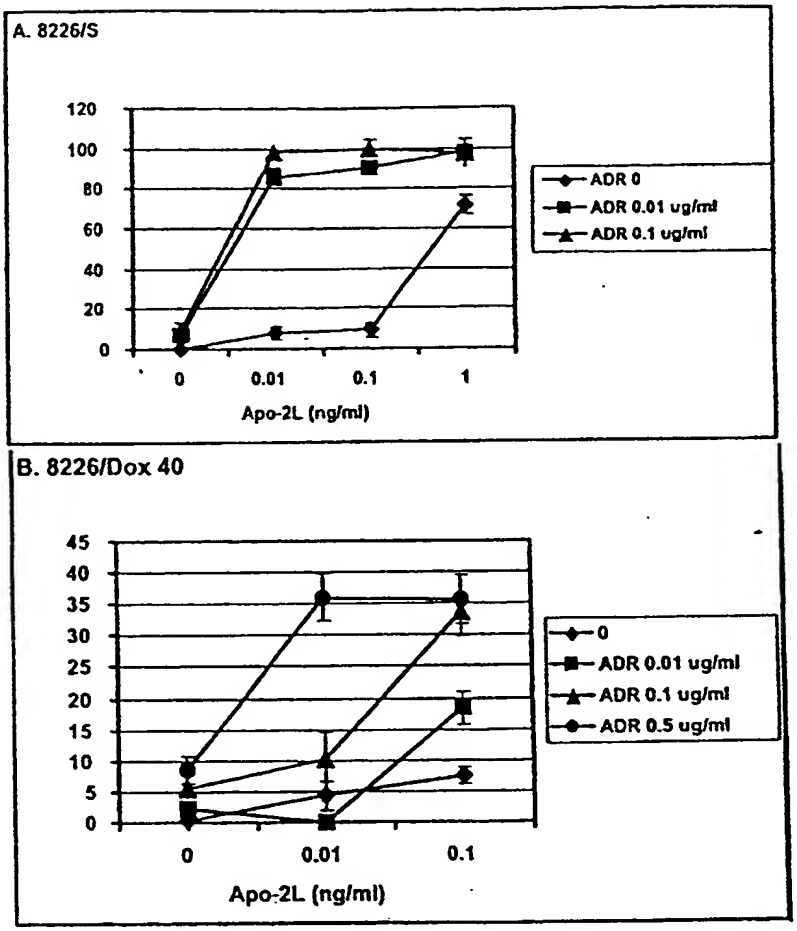

Figure 2. Synergistic cytotoxicity of ADR and TRAIL on 8226 and 8226/Dox 40 human multiple myeloma cell lines (A. 8226; B. 8226/Dox 40). Cells were pre-treated with adriamycin (ADR) for 18 h prior to the experiment. Then soluble TRAIL at the indicated concentrations was added and the percent cytotoxicity was determined by the XTT assay. The results represent the mean ± STD of two separate experiments.

the synergistic killing of TRAIL and drugs in the treatment of drug-resistant human multiple myeloma cell lines. These preliminary results might lead to possible clinical therapeutic approaches in the treatment of drug refractory human multiple myeloma tumor cells.

ii) Prostate cancer. Prostate cancer is one of the most prevalent cancers in American men and the survival rate of patients with advanced prostate cancer is currently low (73). While surgery, hormone therapy, and chemotherapy can eradicate the majority of prostate cancer, relapse of advanced cancer metastasis can occur. The grim prognosis of patients with the advanced disease reflects that the advanced prostate cancer can become unresponsive to current existing therapies. Since the prostate cells that are hormone refractory are also insensitive to radiation therapy and chemotherapy, these cells possibly develop resistance to all apoptotic programs induced by various stimuli as they progress to become more malignant (74,75). Our group has shown that the drug- and hormone-resistant prostate cell lines (e.g. PC3, DU145) are also resistant to immune attacks by cytotoxic immune cells (76,77). Consequently, we have focused our effort on exploring new immunological-based therapeutic approaches to overcome the resistance of advanced prostate cancer cells to apoptosis, such as TRAIL-based cytotoxic therapy.

Recently we have found that subtoxic level of Actinomycin D (Act D) can overcome the resistance of prostate tumor cell lines (DU145, PC3, and LNCaP) to TRAIL-mediated apoptosis. DU145, PC3, and LNCaP are unresponsive to TRAIL-mediated apoptosis, as we have detected less than 5% of cytotoxicity in the cells treated with high concentration of TRAIL (500 ng/ml) for 24 h (data not shown). In sensitive cell lines (such as CEM), 500 ng/ml of TRAIL is sufficient to induce >80% of cell death in 4 h. When these resistant prostate cells are treated with subtoxic level of Act D (50 ng/ml), the cells become sensitive to TRAIL-mediated apoptosis (Table III). Act D can sensitize cells to even low concentration of TRAIL (5 ng/ml). In all three cell lines, we were able to augment the killing by at least 30%. LNCaP cells show the most dramatic increase as the killing was enhanced

Table III. Sensitization of prostate cell lines to TRAIL-mediated apoptosis by Actinomycin D.

Cell line	% Apoptotic cells (sub-G1 population)[a]			
	No TRAIL		+5 ng/ml TRAIL	
	Untreated	50 ng/ml Act D	Untreated	50 ng/ml Act D
DU145	1.6	2.3	2.2	48.1
PC3	1.6	2.1	2.7	35.6
LNCaP	2.1	14.2	9.1	75.8

[a]% Apoptotic cells were determined by flow cytometric reading of propidium iodine (PI)-stained cells. The apoptotic fraction was gated as the population that showed less amount of fluorescence adjacent to the G0/G1 peak. Cells were treated with 50 ng/ml of Act D and 5 ng/ml of TRAIL simultaneously for 24 h. Then, they were harvested, fixed, stained with 50 µg/ml PI and read in the flow cytometer (FL3).

Table IV. TRAIL receptor expression in prostate carcinoma cell lines.

Cell line	Expression of TRAIL receptor mRNA[a]				Sensitivity to TRAIL killing[b]
	DR4	DR5	DcR1	DcR2	
PC3	Strong	Weak	Weak	Weak	Resistant
DU145	Strong	None	Weak	Strong	Resistant
LNCaP	Weak	Weak	Weak	Strong	Resistant

[a]Receptor expression was determined by RT-PCR. The level of expression of receptor mRNA was compared to the mRNA level of GAPDH (None indicates 0-5% of GAPDH; Weak indicates 5-50% of GAPDH; Strong indicates 50%-100% of GAPDH). [b]Resistant indicates <5.0% killing at 500 ng/ml TRAIL.

by almost 60%. The expression patterns of agonistic receptors (DR4, DR5) and decoy receptors (DcR1, DcR2) in DU145, PC3, and LNCaP do not correlate very well with their sensitivity to TRAIL-mediated apoptosis (Table IV) (Ng C-P and Bonavida B, manuscript in preparation). The mechanism of sensitization perhaps involves the regulation of intracellular signaling molecules in the TRAIL-mediated apoptosis pathway (Fig. 1).

iii) Kaposi's sarcoma. Kaposi's sarcoma (KS) is the most common malignancy arising in persons with HIV infection (AIDS-KS). The clinical course of AIDS-KS is highly variable, ranging from minimal disease presenting as an incidental finding, to a rapidly progressive or extensive disease resulting in significant morbidity and mortality (78). Although a number of modalities have been used for 15 years, cure or long-term complete remission from KS is unlikely with the currently available therapeutic modalities (79).

We have reported that AIDS-KS cells are resistant to chemotherapeutic drugs (80). AIDS-KS cells are resistant to killing by chemotherapeutic drugs/NK cells and Fas-induced apoptosis, suggesting that the acquisition of anti-apoptotic characteristics by AIDS-KS cells may contribute to their prolonged survival. Apo-2 ligand (Apo-2L)/TNF-related apoptosis-inducing ligand, a new member of the TNF family, has been identified as an apoptosis-inducing molecule. In this study we examined the sensitivity of 10 different AIDS-KS isolates to Apo-2L-mediated cytotoxicity. AIDS-KS cells were relatively resistant to Apo-2L; however, Apo-2L and Act D used in combination synergistically potentiated the induction of cell death in 9 of the 10 isolates. Furthermore, Act D did not sensitize PBMC or fibroblast cells to Apo-2L (81). Thus, Apo-2L and Act D used in combination may be of therapeutic value in the treatment of AIDS-KS.

Foreman *et al* (82) reported that high levels of Bcl-x are detected in AIDS-KS lesions, and cultured AIDS-KS cells preferentially express Bcl-x_L. These studies suggest that high levels of Bcl-x and Bcl-x_L may lead to prolonged survival of AIDS-KS cells. In our study we show that AIDS-KS spindle cells preferentially express Bcl-x_L, which is markedly reduced by Act D treatment. Down-regulation of Bcl-x_L may be associated with sensitization of AIDS-KS cells by Act D (81). Therefore, our findings *in vitro* showing synergistic cytotoxic activity of sApo-2L in combination with Act D support their use *in vivo* in the therapy of drug-resistant AIDS-KS.

iv) Bladder cancer. Bladder cancers are of the most common cancers in man and the incidence of bladder cancer continues to increase steadily (83). While the overall response rate of patients with bladder cancer to current anti-cancer chemotherapeutic agents has improved, drug resistance and re-occurrence of cancers remain major obstacles in the treatment of bladder cancer. Thus, more effective therapies are needed to overcome drug resistance. For instance, combination treatment with anti-cancer agents and biologic response modifiers have been considered as new means to reverse drug resistance.

Studies from our laboratory demonstrated that treatment with tumor necrosis factor (TNF-α) in combination with anti-cancer drugs resulted in significant potentiation of cytotoxicity and synergy against a variety of sensitive and resistant human bladder cancer cells (84). Likewise, we have reported that doxorubicin sensitizes human bladder cancer cells to Fas-mediated apoptosis (85). We have examined if drugs also sensitize bladder cancer cells to TRAIL-mediated apoptosis. Both established human bladder cancer cell lines and fresh bladder tumor cells were tested. The human T24 bladder cancer cell line was relatively resistant to soluble TRAIL. However, treatment of T24 with combination of TRAIL and ADR resulted in a synergistic cytotoxic effect. Synergy was also achieved in the ADR resistant T24 (T24/ ADR), and two other bladder cancer cell lines. Synergy and apoptosis was achieved also with other drugs like epirubicin and pirarubicin. Freshly-derived human bladder tumor cells were

resistant to TRAIL-mediated cytotoxicity, but can be sensitized by ADR. The concentration of ADR used to sensitize bladder tumor cells was subtoxic (86). These findings demonstrate that the combination treatment with TRAIL and drugs results in synergistic cytotoxicity and apoptosis against both acquired and natural drug-resistant bladder cancer cells. The synergistic effect is not restricted to established cell lines but is also achieved in freshly derived bladder cancer. These findings also point out the potential therapeutic effect of combinations with TRAIL and drugs in the treatment of patients with drug-resistant bladder tumors.

7. Mechanisms of TRAIL-resistance and sensitization

The mechanism of overcoming tumor resistance to TRAIL-mediated apoptosis by drugs or cytokines has not been extensively investigated. However, some key experiments have suggested two major modes of mechanisms by which tumor cells can be sensitized by drugs or cytokines to TRAIL-mediated apoptosis. One is the suppression of anti-apoptotic molecules; another is the upregulation of pro-apoptotic molecules. For example, Bcl-x_L and Bcl-2, major inhibitors of the mitochondrial apoptotic pathway, can be regulated by drugs. Actinomycin D, a drug that inhibits RNA synthesis, has been shown to preferentially decrease the expression of Bcl-x_L and sensitize AIDS associated Kaposi's sarcoma cells to TRAIL-mediated apoptosis (81). In addition, low level of Taxol can reduce the activity of Bcl-2 by inducing the phosphorylation of Bcl-2, which inactivates Bcl-2 function and allows the activation of the mitochondrial pathway (87,88). The active mitochondrial pathway can potentially cross-talk with the TRAIL-mediated pathway and enhances tumor sensitivity to apoptosis. Drugs and cytokines can also upregulate the expression of pro-apoptotic molecules to lower the signaling threshold required for the induction of TRAIL-mediated apoptosis. The expression of DR5, one of the death-inducing TRAIL receptors, has been shown to be inducible by genotoxic drugs and TNF-α (89,90). The induction of DR5 appears to be regulated by both p53-dependent and p53-independent mechanisms (90). In addition, the mRNAs of caspases (caspase-1, -2, -6, -8, and -9) can be upregulated by γ-IFN (91). The upregulation of these caspases can potentially enhance the sensitivity to apoptosis, as γ-IFN has been shown to sensitize tumor cells to TRAIL-mediated apoptosis (92). In summary, these two sensitization schemes, the suppression of anti-apoptotic molecules and the induction of pro-apoptotic molecules, are potential strategies that we can utilize to sensitize resistant cells to TRAIL-mediated apoptosis. The various signaling molecules involved in the TRAIL apoptotic pathways then become attractive therapeutic targets for controlling tumor sensitivity to TRAIL-mediated apoptosis.

8. Concluding remarks

In the last half century of anti-cancer therapy, the principles of drug-induced DNA damage have lead to some impressive clinical results and surprising insights into cell biology. In general, anti-neoplastic therapy consists of cytotoxic drugs designed to induce defects in cell replication and repair. In its most extreme form, hematopoietic progenitor cell transplantation allows for the delivery of dose-intensive cytotoxic chemotherapy. Conventional chemotherapy does not simply prevent cell replication, but in many cases induces a process of programmed cell death. A variety of protective mechanisms are used by malignant cells to confer a phenotype of chemotherapy resistance (93). The induction of apoptosis may not only permit for the development of more effective anti-neoplastic treatment, but hopefully more specific therapy as well (94).

Unfortunately, for many patients with chemotherapy-sensitive malignancy (acute leukemia, lymphoma, multiple myeloma), the greatest advantage for conventional chemotherapy accrues to those patients who are often able to tolerate very intensive and potentially toxic chemotherapy. In only one disease setting, acute pro-myelocytic leukemia, has the use of a pro-apoptotic, differentiating agent, all transretinoic acid, been incorporated into the conventional cytotoxic regimen (95,96). In this setting, toxic side effects have decreased and efficacy has improved thus holding promise for agents that will not only confer enhanced tumor sensitivity to cytotoxic agents but will also be expected to confer an enhanced degree of specificity.

In the next decade, cytotoxic drugs will be combined with biological response modifiers to overcome drug resistance. Given the large number of mutations among malignant cells, pro-apoptotic mechanisms may be relatively specific allowing for more selective cytotoxicity and less collateral toxicity. These new forms of treatment will be studies in new settings and with no doubt, lead to an improved understanding of the role of cell-signaling molecules in the induction of apoptosis and in the management of malignant diseases.

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